Supplementary Methods

Study design

In this study, volunteers from the Escola Paulista de Medicina/UNIFESP and other institutions in São Paulo were recruited between January and November 2021. The participants were healthy, of both sexes, >18 years old, vaccinated with two homologous doses of CoronaVac (CV, n = 25) or ChAdOx1 (Ch, n = 23), and with a third heterologous dose of BNT162b2 (CV/CV/BNT or Ch/Ch/BNT groups). For CV/CV/BNT, blood samples were collected at five time points: 0-7 days before the first dose (t0), 28 days after the first dose and before the second dose (t1), 14 days after the second dose (t2), 75 days after the second dose (t3), and 14 days after the booster dose with BNT162b2 (t4) (Figure 1A). For Ch/Ch/BNT, blood samples were also collected at five time points: 0-7 days before the first dose (t0), 75 days after the first dose and before the second dose (t1'), 14 days after the second dose (t2'), 75 days after the second dose (t3'), and 14 days after the booster dose with BNT162b2 (t4') (Figure 1A).

All participants were tested for anti-S1 IgG at all time points. Neutralizing antibodies and cellular responses were evaluated at t3, t3', t4, and t4'. All individuals enrolled in this study provided written informed consent as part of the protocols approved by the Ethics Committee of the Federal University of São Paulo and by the National Ethics Committee (Comissão Nacional de Ética em Pesquisa) (CONEP, study number CAAE: 32571720.0.0000.5505). The study was conducted in compliance with the principles of the Declaration of Helsinki[1].

Blood collection

Venous peripheral blood sample (10 ml) was collected using standard venipuncture and processed within 3 h in the laboratory. Whole blood samples were used immediately for interferon-gamma (IFN-γ) release assay (IGRA). Plasma samples were obtained after blood centrifugation at 3,000 rpm for 10 min and stored at −80°C for further analysis.

Anti-SARS-CoV-2 antibodies

Quantitative anti-S1 IgG (Anti-SARS-CoV-2 QuantiVac ELISA IgG) and semiquantitative anti-S1 IgA (Anti-SARS-CoV-2 ELISA IgA), anti-NCP IgG (Anti-SARS-CoV-2 NCP ELISA IgG) levels were measured in plasma using Anti-SARS-CoV-2 ELISA kits (EUROIMMUN, Lübeck, Germany), according to the manufacturer's instructions. Results of quantitative anti-S1 IgG are expressed in binding antibody units/ml (BAU/ml): <25.6 BAU/ml were considered negative, ≥25.6 and <35.2 BAU/ml were indeterminate, and ≥35.2 BAU/ml were positive. The results of semiquantitative anti-S1 IgA and anti-NCP IgG titers are expressed as ratios from the extinction coefficient of the sample to that of the calibrator: <0.8 were considered negative, ≥0.8 and <1.1 were indeterminate, and ≥1.1 were positive.

Neutralizing antibodies

The neutralizing antibody levels were determined in plasma using the SARS-CoV-2 NeutraLISA assay (EUROIMMUN) according to the manufacturer's instructions. In this assay, neutralizing antibodies compete with biotinylated ACE-2 for the SARS-CoV-2 spike S1 domain. Results are expressed as the percentage of inhibition (%IH) of ACE-2 binding to S1: <20% was considered negative, ≥20% and <35% were indeterminate, and ≥35% was positive.

IFN-γ assay

Whole blood samples were collected in heparin sample tubes and stimulated with components of the S1 domain (IGRA tube), mitogen (IGRA Stim), or control without activation (IGRA Blank) using the SARS-CoV-2 IGRA Stimulation Tube Set (EUROIMMUN). After 24-h incubation at 37°C ± 1°C, samples were centrifuged at 12,000 g for 10 min, and the plasma was used for the SARS-CoV-2 Interferon Gamma Release Assay (EUROIMMUN). The analyses were performed according to the manufacturer's instructions. Results are expressed in milli-international units per

milliliter (mIU/ml): <100 mIU/ml were considered negative, ≥100 and <200 mIU/ml were indeterminate, and ≥200 mIU/ml were positive.

Statistical analysis

Statistical significance tests were performed using the non-parametric Wilcoxon–Mann–Whitney test. Correlation coefficients between two variables were quantified using Spearman's rank-order correlation. All tests were performed in a two-sided manner using a significance threshold (p) of <0.05, with OriginPro 2016 (OriginLab).

References

1 World Medical Association Declaration of Helsinki. *JAMA* 2013;**310**(20):2191. Doi: 10.1001/jama.2013.281053.